165. The pharmaceutical formulation of claim 163, wherein said antibody is a genetically-engineered monoclonal antibody.

166. The pharmaceutical formulation of claim 165, wherein said antibody is a single-chain antibody.

167. The pharmaceutical formulation of any one of claims 162-166, wherein said beta-amyloid is human beta-amyloid.

REMARKS

Claims 1-4 and 150-167 presently appear in this case. No claims have been allowed. The official action of August 22, 2003, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

The following statements are made pursuant to the requirements of 37 C.F.R. §1.173(c). Patent claims 1-4 are pending. Added claims 5-149 have been cancelled. Claims 150-167 are newly presented in the present amendment. As for an explanation of the support in the disclosure of the patent for the changes made to the claims, reference is made to the attached chart entitled "Support for New Reissue Claims." This chart sets forth examples of support in the disclosure of the patent for each of the claim limitations.

Briefly, the present invention relates to pharmaceutical formulations comprising an antibody or an antigen binding fragment thereof and a pharmaceutically acceptable carrier. The antibody and fragment recognize an

epitope within residues 1-28 of β-amyloid or are obtainable using residues 1-28 of β-amyloid as an immunogen and they inhibit aggregation of β-amyloid or they maintain the solubility of soluble β-amyloid. The antibody is preferably a monoclonal antibody, and more preferably a human monoclonal antibody, a genetically engineered monoclonal antibody, or a single chain antibody. The β-amyloid is preferably human β-amyloid.

The interview among Examiner Nichols, S.P.E. Kunz, attorney Gordon Kit, and the undersigned on February 11, 2004, is hereby gratefully acknowledged. In this interview, the claims submitted herewith were discussed as was the data reported in the declaration of Prof. Beka Solomon attached hereto. Furthermore, the nature of the aggregation assay appearing in the specification was clarified for the examiner. The arguments presented at the interview will be substantially repeated in the discussion of the rejections below.

The official action of August 22, 2003 was a final rejection. Withdrawal of the finality of this rejection, however, is hereby respectfully urged.

MPEP 706.07(a) says:

> Under present practice, second or any subsequent actions on the merits shall be final, except where the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims, nor based on information submitted in an information disclosure statement filed during the period set forth in 37 C.F.R. 1.97(c) with the fee set forth in 37 C.F.R. 1.17(p).

In the Official action of August 22, 2003, claim 126 was rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Claim 126 is substantially the same as previously appearing claim 20. However, in the previous Official action on the merits of June 29, 2001, claim 20 was not made the subject of a 35 U.S.C. §112 rejection. It is apparent that the present rejection under 35 U.S.C. §112 would have been equally applicable to previously appearing claim 20, so it is clear that this rejection was not necessitated by applicant's amendment. Thus, in accordance with the present policy the Patent and Trademark Office as set forth in the above-quoted portion of the MPEP, the finality of this Official action was premature. Reconsideration and withdrawal thereof is respectfully urged.

It is noted that in the interview of February 11, 2004, the examiners agreed that the finality of the Official action of August 22, 2003, would be withdrawn.

On January 22, 2004, a notice of appeal was filed in this case. Regardless of the finality of the Official action of August 22, 2003, the notice of appeal is effective because this is at least the second Official action on the merits. In view of the withdrawal of the finality of the Official action of August 22, 2003, the present amendment should be entered as a matter of right and appropriately responded to. Thus, applicant has elected to continue prosecution, as is permissible in view of the withdrawal of the finality of the

previous Official action, rather than to continue with the appeal.

The examiner has objected to the specification because there is a typed correction next to the abstract as filed ("[molecules]") and then typed next to this is "involves" typed over something covered in whiteout. The examiner states that this correction or alteration has not been entered as an amendment.

While it is believed that this type of amendment to the abstract is in full accordance with 37 C.F.R. §1.173(b) and (d), nevertheless the amendment to the abstract is being re-presented by the present amendment. It is believed that this objection has now been obviated and the correction to the abstract may now be entered.

The examiner states that the original patent, or a statement as to loss or inaccessibility of the original patent, must be received before this reissue application can be allowed.

Submitted herewith is the original Letters Patent with respect to patent no. 5,688,651. Accordingly, the requirement of 37 C.F.R. §1.178 has been met and the present application can proceed to allowance.

Claims 1-4 and 126-149 have been rejected as being based upon a defective reissue declaration. The examiner states that a supplemental reissue declaration must be received before the reissue application can be allowed. The examiner states that receipt of an appropriate supplemental

declaration under 37 C.F.R. §1.175(b)(1) will overcome this rejection under 35 U.S.C. §251.

Attached hereto is a supplemental declaration under 37 C.F.R. §1.175(b)(1). Accordingly, this rejection has now been obviated. As this was the only rejection of claims 1-4, these claims should now be considered to be in condition for allowance.

In the Official action of August 22, 2003, the examiner stated that claims 126-149 were rejected under 35 U.S.C. §251 for lack of defect or error in the original patent, and as not being an error correctable by reissue. Pursuant to a telephone interview of August 25, 2003, an interview summary form was issued correcting this line of the Official action, and confirming that "in fact only claims 130-149 are rejected." The summary record goes on to state that the first line of paragraph 12, page 3 of the final rejection of August 22, 2003 should read, "Claims 130-149 are rejected under 35 U.S.C. §251..."

Claims 130-149 have now been deleted without prejudice toward the continuation of prosecution thereof in a continuing application. Accordingly, this rejection has now been obviated.

Claims 126-129 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. This rejection is respectfully traversed.

At paragraphs 18 and 19, the examiner questions whether the "denatured" CPA protein is in fact "aggregated," and questioned whether the data supports the claims. In this regard, it should be noted that the present claims do not cover monoclonal antibodies specific for CPA, as the present claims are all directed to antibodies and fragments thereof that recognize an epitope of β-amyloid. It should be noted for the record, however, that it is aggregation, which causes the CPA protein to become denatured. Note the present specification at column 9, lines 49-52 and 57; column 10, line 52; column 12 lines 53-57; and column 13 line 53.

In paragraph 20 of the Official action, the examiner contends that there is no evidence in the Solomon application or Solomon (PNAS 1996)[1] of prevention of Aβ aggregates because the assay includes the step of removing aggregates.

The examiner appears to have misunderstood the assay technique employed. More specifically, in the assay, the test solution (containing a fixed and predetermined amount of Aβ alone, the same amount of Aβ in combination with heparan sulfate, the same amount of Aβ in combination with Al^{+++}, or the same amount of Aβ in combination with Zn^{++}) was heated for 3 hrs at 37°C (which is the physiological temperature). This heat treatment results in the formation of aggregates of Aβ. Next, the aggregates of Aβ were removed by centrifugation, and the supernatants (containing any remaining soluble Aβ) were

[1] Solomon et al, Proc Natl Acad Sci USA 93:452 455 (1996)

incubated for 60 min with excess AMY-33 (a mouse monoclonal antibody raised against amino acids 1-28 of Aβ) to produce immunocomplexed soluble Aβ. Then, an ELISA was carried out by adding the resulting supernatant (containing the immunocomplexed Aβ) to microtiter plates that had been pre-coated with rabbit anti-Aβ antibody, resulting in the binding of any immunocomplexed Aβ in the supernatant to the plates. Next, immunocomplexed Aβ bound to the plate was measured using HRP-labeled goat anti-mouse antibody, which binds to AMY-33 of the immunocomplex, and degradation of the substrate O-phenylenediamine by the HRP was monitored by OD. As discussed below in the context of paragraph 22 of the Official action, this represents a quantitative measurement of soluble Aβ remaining after removal of the aggregate, and, by simple subtraction from the starting amount, the total amount of aggregate that was remaining.

As shown in Figure 1A of Solomon (PNAS 1996) and Figure 7A of the Solomon application:

(1) using only an aqueous solution of Aβ (1-40), the OD was about 0.1, i.e., there was not much soluble Aβ in the supernatant, and hence the conditions induced aggregation.

(2) using an aqueous solution of Aβ (1-40) containing heparan sulfate, the OD was about 0.02, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Heparan sulfate is taught in the Solomon application and Solomon (PNAS 1996) to be associated with "aggregation of pre-existing fibrils."

(3) using an aqueous solution of Aβ (1-40) containing Al^{+++}, the OD was about 0.03, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Al^{+++} is merely taught in the Solomon application and Solomon (PNAS 1996) to be "proposed as a 'risk factor' for Alzheimer's disease".

(4) using an aqueous solution of Aβ (1-40) containing Zn^{++}, the OD was about 0.02, i.e., not much soluble Aβ in the supernatant, and hence the conditions induced aggregation. Zn^{++} is also merely taught in the Solomon application and Solomon (PNAS 1996) to be "proposed as a 'risk factor' for Alzheimer's disease".

In a parallel set of experiments, monoclonal antibody AMY-33 was added to each sample before the first incubation, i.e., before induction of aggregation, so as to produce immunocomplexed soluble Aβ before induction of aggregation. In this manner, prevention/inhibition of aggregation was measured.

As shown in Figure 1A of Solomon (PNAS 1996) and Figure 7A of the Solomon application:

(1) using AMY-33 and only an aqueous solution of Aβ (1-40), the OD was about 0.54, i.e., there was a large amount of soluble Aβ in the supernatant, and hence a lot of prevention of aggregation.

(2) using AMY-33 and an aqueous solution of Aβ (1-40) containing heparan sulfate, the OD was about 0.65, i.e.,

there was a large amount of soluble Aβ in the supernatant, and hence a lot of prevention of aggregation.

(3) using AMY-33 and an aqueous solution of Aβ (1-40) containing Al^{+++}, the OD was about 0.04, i.e., not much soluble Aβ in the supernatant, and hence not much prevention of aggregation.

(4) using AMY-33 and an aqueous solution of Aβ(1-40) containing Zn^{++}, the OD was about 0.08, i.e., not much soluble Aβ in the supernatant, and hence not much prevention of aggregation.

Thus, the evidence in the Solomon application and in Solomon (PNAS 1996) shows that aggregation can be prevented/inhibited using AMY-33, an antibody raised against amino acids 1-28 of Aβ.

Comparable anti-aggregation experiments were carried out using monoclonal antibody 6F/3D, which was raised against amino acids 8-17 of Aβ, and whose epitope maps at amino acids 9-14 (Matsunaga et al (2002)[2]), the results of which are shown in Figure 7B of the Solomon application and Figure 1B of Solomon (PNAS 1996). As shown therein, this antibody did not significantly prevent/inhibit aggregation.

Hanan (1996)[3] confirms the results in the Solomon application and Solomon (PNAS 1996). That is, when using the same heat-induced aggregation assay and antibodies 10D5 and 6C6 (both raised against amino acids 1-28 of Aβ (Bard et al

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[2] Matsunaga et al, Biochem J 361(Pt 3):547-56 (2002)
[3] Hanan et al, Amyloid: Int J Exp Clin Invest 3:130-133 (1996)

(2003)[4]; 2H3 (raised against amino acids 1-12 of Aβ), and 1C2 (raised against amino acids 13-28 of Aβ), it was found that antibodies 10D5 and 6C6 were most effective at preventing/inhibiting the formation of aggregates (see Figure 1 thereof).

Moreover, the electron micrographs of Figure 2 of Solomon (PNAS 1996) clearly demonstrate that AMY-33 converts fibrillar Aβ to an amorphous state, and prevents/inhibits aggregation. Similarly, the electron micrographs of Figure 1 of Solomon (Fisher 1998)[5] confirm these results using 6C6 (raised against amino acids 1-28 of Aβ), i.e., this antibody also prevents/inhibits aggregation.

Solomon (PNAS 1997)[6] confirms the results in the Solomon application and Solomon (PNAS 1996). That is, when using a similar assay (but that measures disaggregation), and antibodies 6C6 (raised against amino acids 1-28 of Aβ; (Bard et al (2003)); 1C2 (raised against amino acids 13-28 of Aβ), and 14C2 (raised against amino acids 33-40 of Aβ), it was found that antibody 6C6 was most effective at solubilizing Aβ (see Figure 1 thereof).

In paragraph 21 of the Official action, the examiner notes that 6F/3D showed no discernable effect on prevention of Aβ aggregates.

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[4] Bard et al, Proc Natl Acad Sci USA, 100:2023-2028 (2003)
[5] Solomon et al in Progress in Alzheimer's and Parkinson's Diseases, edited by Fisher et al, Plenum Press, New York, 205-209 (1998)
[6] Solomon et al, Proc Natl Acad Sci USA 94:4109-4112 (1997)

However, the 6F/3D antibody does not fall within the scope of the claims because it does not inhibit aggregation. The claims all require that the antibody inhibit aggregation. Thus, it would not be expected to have any discernable effect on prevention of Aβ aggregates.

Also, in paragraph 21 of the Official action, the examiner notes that AMY-33 did not show an inhibitory effect on metal Al- or Zn-induced aggregation. The examiner contends that since Al and Zn are present in physiological conditions, these results cast doubt on the *in vivo* utility of AMY-33.

The assay in Example 2 is discussed in detail above. With this better understanding of the assay it can be seen that it is not accurate to refer to "metal-induced" beta-amyloid aggregation. In fact, aggregation of beta-amyloid in the assay was induced using heat, i.e., 37°C. The assay was carried out under three conditions, (a) heat alone, (b) heat in the presence of Zn^{++} and (c) heat in the presence of Al^{+++}. The assay does not employ "metal-induced" aggregation *per se* as apparently contended by the examiner.

It should be understood that Zn^{++} is merely one of many factors that are "speculated" in the present application as a risk factor for Alzheimer's disease. Recent evidence has suggested that 100 μM Zn^{++} actually has a protective effect against Aβ toxicity (Yoshiike et al (2001)[7]).

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[7] Yoshiike et al, J Biol Chem 276:32293-32299 (2001)

Furthermore, Al^{+++} is another factor that is merely "speculated" in the present Application as a risk factor for Alzheimer's disease. Indeed, Al^{+++} has no known physiological function (Trombley (1998)[8]).

The data in the present Application with respect to the contribution of Zn^{++} and Al^{+++} is simply inconclusive. Thus, contrary to the examiner's contention, the assay results in the presence of Al^{+++} and Zn^{++} do not cast doubt on the *in vivo* utility of AMY-33, whose results in the heat-induction assay are clear, and supported by subsequent *in vitro* and *in vivo* tests.

The examiner's attention is also invited to the attached declaration of Prof. Beka Solomon, reporting on an experiment that was conducted to show the correlation between positive results in the heat-induced aggregation assay, in the absence of Zn or Al, with positive *in vivo* results. Prof. Solomon reports on a repetition of the experiment in example 2 of the present specification, using the AMY-33 antibody as well as the 10D5 antibody. The results show that antibody 10D5 is effective in inhibiting heat-induced aggregation in the absence of Zn and Al, but it is not very effective in inhibiting heat-induced aggregation in the presence of Zn or Al. In this regard, the results are similar to the results shown with the AMY-33 antibody. The results for the AMY-33 antibody are consistent with the results reported in the

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[8] Trombley, J Neurophysiol 80:755-761 (1998)

specification of the reissue application. Both AMY-33 and 10D5 are monoclonal antibodies raised using amino acids 1-28 of β-amyloid as an immunogen. Both have been shown to maintain the solubility of soluble β-amyloid.

10D5 antibody has been shown to reduce pathology in a mouse model of Alzheimer's disease, and to cause clearance of plaques *in vivo* in a mouse model of Alzheimer's disease. It has also been reported to be effective at suppressing Aβ deposition and to act as an Aβ sink *in vivo* (see DeMattos et al (2001)[9]).

This declaration establishes that the results of the heat-induced aggregation assay in the absence of Zn and Al are the most relevant to predicting *in vivo* activity. Accordingly, it would be expected that additional antibodies, which are raised using amino acids 1-28 of β-amyloid as the immunogen, or which otherwise recognize an epitope within residues 1-28 of β-amyloid, and which inhibit heat-induced aggregation in the absence of Zn and Al, as set forth in the above-identified reissue application, would be active *in vivo* notwithstanding the results of the heat-induced aggregation assay in the presence of Zn or Al.

The examiner's attention is also directed to Figure 3 of Solomon (PNAS 1996), and Figure 2 of Solomon (Fisher 1998), and Figure 2 of Solomon (PNAS 1997). These experiments confirm the above-discussed results.

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[9] DeMattos et al, Proc Nat Acad Sci USA 98:8850-8855 (2001)

Figure 3 of Solomon (PNAS 1996) shows that soluble Aβ has no effect on the florescence of the dye Thioflavin T, whereas aggregated Aβ changes the excitation spectrum of Thioflavin T. Adding AMY-33 to soluble Aβ, prior to aggregation (incubation at 37°C), prevented the change in florescence, i.e., prevented/inhibited aggregation.

Figure 2 of Solomon (Fisher 1998) shows that soluble Aβ has no effect on the florescence of the dye Thioflavin T, whereas aggregated Aβ changes the excitation spectrum of Thioflavin T. Adding 6C6, 10D5, 2H3, 1C2, or 266 to soluble Aβ, prior to aggregation (incubation at 37°C), prevented the change in florescence, i.e., prevented/inhibited aggregation.

Figure 2 of Solomon (PNAS 1997) shows that anti- Aβ antibodies disrupt Aβ fibrils. Fibrils of Aβ were first formed, and then incubated with 6C6 or IC2. 6C6 was found to extensively disrupt fibrils, whereas IC2 was found to only slightly interfere with fibril disaggregation.

In paragraph 22 of the Official action, the examiner contends that the assays preformed measure A495 (OD) or fluorescence, which are relative and not quantitative measurements.

Contrary to the examiner's contention, Hanan (1996) shows that the OD data was concentration dependent (see Figure 1, insert), and thus a quantitative measurement. Further, the fluorescence was concentration dependent (see e.g., Figure 2 of Solomon (Fisher 1998) and the legend of Figure 2 of Solomon (PNAS 1997)), and thus a quantitative measurement.

In paragraph 23 of the Official action, the examiner contends that the specification and the prior art do not provide any support to correlate the prevention, disaggregation or inhibition of aggregation with an alleviation of symptoms or providing some relief to the patient.

The examiner is requested to note that the PDAPP mouse has been recognized in the art as being a major breakthrough in the production of an animal model for Alzheimer's disease. The importance and breakthrough nature of the PDAPP mouse is evident, i.e., it was a cover story in *Nature* in 1995 (Games et al (1995)[10]). The PDAPP transgenic mouse described in Games et al (1995) exhibit age- and brain region-dependent development of typical amyloid plaques, dystrophic neurites, loss of presynaptic terminals, astrocytosis and microgliosis. These lesions in the PDAPP mouse brain tissue are typical of many of the neuropathological hallmarks associated with Alzheimer's disease. Games et al (1995) also teaches that in the PDAPP mice, neurodegeneration and inflammation characteristic of Alzheimer's disease, with associated Aβ plaque deposition and certain regions of afflicted brain parenchyma, are present. Deposition of brain deposits in the PDAPP mice increases with age, as is found in Alzheimer's disease. Thus, the PDAPP

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[10] Games et al, Nature, 373:523-527 (1995)

mouse shows much of the pathology seen in Alzheimer's disease patients.

Games et al (1995) concludes, at page 527, second paragraph, first column:

> A most notable feature of these transgenic mice is their Alzheimer-like neuropathology .... Our transgenic model ... offers a means to test whether compounds that lower Aβ production and/or reduce its neurotoxicity *in vitro* can produce beneficial effects in an animal model prior to advancing such drugs into human clinical trials.

Similarly, Schenk et al (1999),[11] which was a cover story in *Nature* in 1999, concludes, at page 177, paragraph bridging columns 1 and 2:

> To our knowledge, this is the first report of a clinically relevant treatment that reduces the progression of AD-like neuropathology in a transgenic model [the PDAPP mouse] of the disease .... Collectively, the results suggest that amyloid β immunization may prove beneficial for both the treatment and prevention of Alzheimer's disease.

Thus, Games et al (1995) and Schenk et al (1999) teach that the PDAPP mouse exhibits many of the pathological characteristics of Alzheimer's disease, and is regarded in the art as a model reasonably predictive of results in humans.

As shown in Bard et al (2003); Bard et al (2000);[12] and Bacskai et al (2001),[13] *inter alia*, antibodies 6C6 and 10D5 (again both raised against amino acids 1-28 of Aβ) were

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[11] Schenk et al, Nature 400:173-177 (1999)
[12] Bard et al, Nature Medicine 6:916-919 (2000)
[13] Bacskai et al, Nature Medicine 7:369-372 (2001)